Report on Research Activities of the

Lt. J. P. Kennedy, Jr. Laboratories for Molecular Medicine

Stanford University School of Medicine

July 1, 1963 - June 30, 1964

For the most part, this report concerns research in the departments of Genetics and Pediatrics, some of which has been furthered by allocations from the Fluid Research Fund of the Laboratories.

Kennedy Laboratories Fluid Research Fund

Allocations during 1963-64

Total amount forwarded - 1963	$25,000
Sub-allocation to Dr. Guy McKhann, Pediatrics Department	$ 4,500
Laboratory equipment and salary of laboratory technician to set up a pediatric neurology service.	
Sub-allocation to Dr. Luigi Luzzatti, Pediatrics Department	$ 4,000
Laboratory equipment and salary of laboratory technician for a cytogenetic laboratory to evaluate the relationship between chromosomal abnormalities and congenital anomalies.	
Sub-allocation to Dr. Jack Remington, Palo Alto Medical Research Foundation	$ 4,500
Study by Dr. Remington and associates in San Salvador to evaluate possible significance of Toxoplasmosis in mental deficiency, premature birth, early postnatal fetal mortality, congenital malformation, abortion.	
Sub-allocation to Dr. Norman Kretchmer, Pediatrics Department	$ 2,000
Cell culture laboratory	
Allocation for use in census project, collection and analysis of data from Pacific State Hospital, California Public Health Service, U. S. Census Bureau; Dr. J. Lederberg and Dr. Walter Bodmer, Genetics Department.	$10,000

July 1, 1964
Stanford University School of Medicine

August, 1964

In the interval since the previous report to the Lt. Joseph P. Kennedy Jr. Foundation (August, 1963) there has been expansion of the activities relating to the study and care of patients with mental retardation and other problems of the developing nervous system. This increased activity has taken place in the areas of personnel, clinical service, teaching, community activities, and research.

PERSONNEL

In the previous report, it was mentioned that the major problem was one of adequate personnel. This problem has been solved by the addition of the following personnel:

1) Dr. Paul Altrocchi. Dr. Altrocchi received his neurology training at the New York Neurological Institute under Drs. Houston Merritt and Sidney Carter. Dr. Altrocchi is an excellent clinician and clinical teacher and is actively participating in the clinical program in neurological diseases and mental retardation.

Dr. Altrocchi's research interests are primarily in the field of the diseases of muscle. At present, Dr. Altrocchi, in collaboration with Dr. A. Kent Christensen of the Department of Anatomy at Stanford, is utilizing the techniques of electron microscopy and histochemistry to study the normal development of muscle in comparison with patients with muscular dystrophy and Werdnig-Hoffmann's Disease.

2) Dr. Thomas Forrest. Dr. Forrest, a board-certified pediatrician, has taken additional training in pediatric neurology. Dr. Forrest's particular area of interest is the disorders of learning in children. Dr. Forrest is in the process of setting up a controlled study of teaching techniques for children with mental retardation and reading disabilities. The outline of this study is enclosed later in this report.

3) Dr. Gene Phillips. (on leave of absence to the National Institutes of Health). Dr. Phillips completed a pediatric residency, and has spent an additional three years in the study of the normal psychological development of the newborn. Dr. Phillips plans to continue these studies at the N.I.M.H. for two years.

4) Dr. Marshal Klaus. Dr. Klaus has recently joined the Department of Pediatrics to take over the direction of the Premature Research Center. Dr. Klaus is a recognized authority in

the field of pulmonary function of the newborn infant. Dr. Klaus is planning to expand his activities to include the adaptation of the techniques for studying pulmonary function to the study of cerebral blood flow and cerebral oxygen consumption in the newborn infant.

5) Dr. George Hexter. Dr. Hexter, a member of the Department of Psychiatry, is collaborating with the members of the Department of Pediatrics in the teaching and study of the clinical problems relating to mental retardation. This collaboration has been extremely helpful in the development of a unified approach to both the mentally retarded child and to his family.

6) Dr. John Belz. Dr. Belz, a member of the Department of Psychiatry, has been active in the teaching of medical students during the Child Health Clerkship. Dr. Belz has instituted an extremely successful teaching experience for undergraduate students built around visits to normal nursery schools, the Children's Health Council (retarded children), and the Peninsula Children's Center (emotionally disturbed children).

CLINICAL SERVICE

1) Stanford In-Patient Service. Patients with neurological disease and mental retardation continue to constitute 25-30% of the acute in-patient service (10-12 beds). Besides the teaching and care aspects of these patients, many clinical problems arise that provide a stimulus for further laboratory investigations.

2) Stanford Out-Patient Service. Patients with neurological diseases and mental retardation are seen in the general pediatric clinic, pediatric neurology clinic, pediatric seizure clinic, and birth defects clinic. In association with the Pediatric Neurology Clinic, a Pediatric Neurology Conference has been established.

3) Stanford Convalescent Home. The Convalescent Home is now an integral part of the Pediatric Service. Regular pediatric neurology rounds are made at the Convalescent Home, with full-time coverage by a neurology or pediatric resident rotating through pediatric neurology.

4) Children's Health Council. Dr. Forrest is functioning as neurological consultant to the Children's Health Council. His presence there has stimulated interest in trying new teaching approaches and new methods of evaluation.

5) Stanford Aphasia Institute. Under the direction of

Dr. Jon Eisenson, the Aphasia Institute studies older children with problems of language expression and comprehension. The combined study of these patients by Dr. Eisenson, the Pediatric Neurology Service, and electroencephalography has delineated a group of children with congenital and acquired defects of what would normally be their dominant hemispheres. The use of long-term anticonvulsant therapy, despite the lack of overt seizure disorders, is under investigation.

6) The Premature Research Center. This center is a clinical laboratory for the study of premature infants. The application of this facility to the study of the developing nervous system is indicated by Dr. Gilbert Frank's studies of Circadian Rhythms. (vide infra).

This unit also is providing us with a continuing supply of well-studied premature infants. The long-term study of the physical, mental, and emotional development of these children will be particularly important.

TEACHING

Medical Students

The neurological sciences course given to third year (out of five year) medical students in a combined program integrating neuroanatomy, neurophysiology, neuropharmacology and the basic aspects of neurology and behavioral sciences. Particular emphasis is placed on the developing nervous system. Several lectures are dedicated to the application of present knowledge about the nervous system to the study of developmental defects and degenerative diseases of the nervous system.

In the spring quater a course in selected topics in neurochemistry is given. During this course the various investigators working on the chemistry of the nervous system acquaint the medical students with their areas of investigations and indicate the direction of future research.

In the fourth year, during the Child Health Clerkship, students rotate through the previously mentioned clinical services. In addition, specific lectures relating to the problems of the developing nervous system are given.

Both the pediatric and neurology residents now rotate through the Pediatric Neurology Service. This concentrated exposure has had a marked effect on the neurological sophistication of the pediatricians and pediatric sophistication of the neurologists. This year, the psychiatry residents will rotate through pediatric neurology on an elective basis.

Drs. Altrocchi and McKhann now consult regularly with the Porterville State Hospital. In addition, the residents on the Pediatric Neurology rotation spend two weeks at Porterville studying the wealth of clinical material at this institution.

COMMUNITY ACTIVITIES

During the past year, several members of the Department of Pediatrics gave outside lectures relating to problems of the developing nervous system.

Dr. Norman Kretchmer.

1) "Genetic Aspects of Mental Retardation"
   Michael Reese Hospital, Chicago. February, 1964.
2) "Whither Birth Defects - ?"
   Annual Meeting of the National Foundation, Miami, April, 1964.
3) "Incidence of Mental Retardation in Santa Clara County-Assessing Diagnostic and Treatment Requirements." Mental Retardation Evaluation Clinic Symposium, Palo Alto. June, 1964.
4) "New Discoveries in Mental Retardation"
   University of California School of Nursing, San Francisco. July, 1964.
5) "Hazards of Prematurity"
   Fresno County Hospital, Fresno, California. August, 1964.
6) "The Biochemical Basis of Genetic Diseases"
   Santa Clara County Hospital Post-Graduate Day, San Jose, California. May, 1964.

Pending

1) "Biochemical Basis of Early Detection of Mental Retardation"
   Academy of Pediatrics, New York. October, 1964.
2) "Regional Maturation of the Nervous System in Early Life"
   Conference Participant, Paris. December, 1964.

Dr. Guy M. McKhann

1) "The Degenerative Diseases of the Nervous System,"
   Porterville State School, Porterville, California. October, 1963.
2) "Cerebral Palsy - ?"
   United Cerebral Palsy - Regional Meeting, Sacremento, California. November, 1963.
3) "Metachrometic Leukodystrophy"
   Joseph P. Kennedy Jr. Foundation Symposium on Mental Retardation, New York. February, 1964.

4) "Metabolic Studies of the Leucodystrophies," University of California Medical School, San Francisco. February, 1964.

5) "Biochemical Disorders Affecting the Central Nervous System," The Children's Hospital of Philadelphia, Philadelphia. June, 1964.

Pending

1) "Biochemical Studies of Myelinization" Conference on Regional Maturation of the Nervous System in Early Life, Paris. December 1964.

LABORATORY INVESTIGATIONS

In the interval since the previous report, several research projects relating to the problem of mental retardation have been successfully completed or have yielded promising preliminary results. Drs. Remington, Luzzatti, Cahn and McKhann received direct support for these investigations from the Kennedy Grant to Stanford University. The projects of these investigators will be reported first.

1) The Relationship of Toxoplasmosis to Mental Retardation. Dr. Remington.

A controlled study of patients at the Sonoma State Hospital has been performed, comparing the incidence of positive Toxoplasma skin tests and serological tests in patients with non-specific mental retardation and those with a recognized syndrome(Down's Syndrome). This study has been completed and indicates that Toxoplasmosis is a significant etiological factor in mental retardation. Of interest is the finding that many of the children with serological evidence of Toxoplasmosis did not have the classical clinical picture of chrioretinitis, microcephaly, and intracranial calcifications.

To establish the true incidence of Toxoplasmosis, serological tests are being performed on the entire population of the country of El Salvador. As a part of this study, attempts at isolation of the Toxoplasma organism are being performed on children dying in the neonatal period.

2) The Relationship of Chromosomal Defects to Multiple Congenital Defects. Dr. Luzzatti.

Screening of children with multiple congenital anomalies has continued. In the past year, several new syndromes associated with chromosomal defects have been identified. In selected patients with mongolism, genotypic analysis of the parents and siblings have been performed in an attempt to obtain further information about the mechanism

of the chromosomal abnormality. In addition, studies to associate specific biochemical genetic markers with abnormal chromosomal patterns have been undertaken.

3) Tissue Culture Laboratory. Dr. Cahn.

Funds from the Kennedy Foundation have been used to set up a laboratory for tissue culture. The major emphasis in this laboratory will be the study of enzymatic patterns as a function of maturation. Other current projects utilizing tissue culture techniques are the study of myelinization in tissue culture, techniques of growing fetal brain, and the study of recombination, or self-recognition, of nervous tissue in vitro.

4) Biochemical Studies of Myelinization. Dr. McKhann.

The study of the synthesis of lipids which are components of the myelin sheath has continued. An in vitro system for the synthesis of sulfatides has been worked out. In this system, the role of cerebroside as a precursor of sulfatide has been established. The properties of "cerebroside sulfokinase", the enzyme which converts one myelin lipid to another, are under investigation.

Studies of the maintenance of myelin have been initiated. The enzymatic breakdown of radioactively-labelled myelin in normal animals, animals with allergic encephalomylitis, and animals exposed to radiation is under investigation. These studies have been initiated in an attempt to find a model system comparable to the human leukodystrophies. Investigation of the human leukodystrophies is continuing, particularly the metabolism of radioactive sulfate in patients with metachromatic leukodystrophy.

In addition to the programs supported by the Kennedy Foundation, several other studies relating to mental retardation, on both the clinical and laboratory level, have yielded interesting results, or suggested new approaches to old problems.

1) The Development of Circadian Rhythmicity in Premature Infants. Drs. Frank and Schafer.

The londitudinal study of premature infants indicates the sequential appearance of diurnal patterns of wakefulness, temperature regulation, body movement, and electroencephalographic activity. Preliminary findings suggest that the normal appearance of these diurnal patterns is delayed in the neurologically damaged premature infant.

2) Tryptophan Metabolism in Phenylketonuria. Dr. Greenberg.

The effect of high phenylalanine levels on tryptophan meta-

bolism in animals and in patients with phenylketonuria is under investigation. The possible beneficial effect of altering tryptophan metabolism, as well as lowering phenylalanine levels, in the therapy of phenylketonuria is under consideration.

3) The Application of Computer Techniques to the Study of Convulsive Disorders. Dr. Morrell.

Children with severe convulsive disorders are evaluated by implanted dural electrodes. The symmetry of electrical activity, and the response to evoked potentials, elicited by auditory or visual stimuli, are evaluated on the computer. Preliminary studies suggest that these methods give a better localization of an epileptogenic foci and a more accurate impression of the physiological states of the surrounding normal brain than conventional electroencephalographic techniques. These findings may be helpful in selecting patients for neurosurgical therapy.

4) The Study of the Effect of a Retarded Child in the Family. Dr. Williams.

At the San Mateo Community Hospital, a team consisting of a pediatrician, neurologist, and psychiatrist have initiated a study of the effect of a retarded child on intrafamilial relationships. This study dyals with not only individual families, but also the evaluation of the existing facilities available to such families for support and guidance.

5) The Evaluation of Teaching Techniques in Mentally Retarded Children. Dr. Forrest.

A controlled study of various teaching techniques has been instituted. Children at the Children's Health Council are evaluated in terms of their educational strengths and weaknessess. The long-term effects of standard teaching techniques versus teaching methods adapted to each individual child are being evaluated.

6) Studies of Nerve Growth-Promoting Factor. Dr. Greenberg.

Nerve growth-promoting factor (N.G.F.) exerts a regulatory influence on the growth of the sympathetic nervous system. Recent studies indicate that autonomic ganglia respond to N.G.F. only during a particular period of embryogenesis. The mechanisms of N.G.F. action, both in terms of its selective action on a particular nervous system at a specific time and its effect on protein synthesis as a reflection of growth, are under investigation.

7) Urea Biosynthesis in the Central Nervous System. Drs. Doherty and Kretchmer.

Mental retardation is associated with three different biochemical errors in the metabolism of ammonia. The role of the urea cycle in normal and abnormal neuronal functioning is under investigation.

8) The Electron Microscopy and Histochemistry of Developing Muscle. Drs. Altrocchi and Christensen.

Of the various tissues affected by genetically determined diseases, muscle is one of the most accesible. Studies are in progress of the normal morphological and chemical development of muscle, to serve as a baseline for the study of specific disease entities.

9) Metabolic Screening of Patients with Mental Retardation. Pediatric Department.

Routine screening of the urine, serum, and cerebrospinal fluid for amino acids, mucopolysaccharides, sugars, and lipids is performed on patients with mental retardation. These procedures have turned up previously undiagnosed cases of well-known entities such as phenylketonuria, histidinemia, and metachromatic leucodystrophy as well as previously undescribed metabolic defects which are currently being investigated.

STUDIES IN THE DEPARTMENT OF GENETICS

Demographic Studies on Mental Retardation and Family Structure.

Drs. J. Lederberg and W. Bodmer.

With the cooperation of Dr. George Tarjan, the entire file of case records of Pacific State Hospital, Pomona, California, is being transferred to punched cards and magnetic tape for comprehensive statistical analysis. It is hoped that these studies will allow a more precise definition of the identity and characteristics of the institutionalized mentally retarded than has been available so far.

In view of the reports by Knobloch and Pasamanick on an unusual incidence of season of birth among mentally retarded in Ohio institutions, the Pomona file was very carefully studied with respect to this variable. Although the first analysis seemed to corroborate such a discrepancy, most of this was found to be traceable to the unreliability of certain records, mostly of some antiquity. When the unreliable records were culled, no striking anomaly in the distribution of season of births could be found in the retarded population.

A more detailed analysis of the variation of distribution of birth season shows a considerable variation in seasonality with the socio-economic or occupational status of the parents. Since institutionalized children in hospitals for the mentally retarded are by no means a random sample of the population, this may also make an important contribution to discrepant season distributions. Further studies are being undertaken to determine, if possible, the factors underlying the variations in birth seasonality in different population groups, and to determine whether these may play some role in differential incidence of mental retardation.

In this connection, it has been found necessary to obtain more reliable tabulations than have been available so far for the control population.

Fortunately, we have been able to obtain the active cooperation of the Bureau of the Census and arrangements are in progress for stationing a representative of the Bureau at Stanford University for very detailed demographic studies of public health interest. Since the order of birth has considerable interaction with the incidence of prematurity and has been suspected to play an important role in intellectual performance children, these factors are being especially closely studied.

A preliminary analysis of data from the state of California suggested that children who were born on weekends might suffer an unusual risk of birth injury, which could be traceable to varying standards of obstetrical care during regular and irregular work shifts at the hospitals. This effect is definitely supported by studies on the incidence of reports of birth injury on birth certificates. However, on a much larger sample they were not substantiated by a statistical analysis of neonatal deaths, which should be a more precise, objective measure of the risk of birth injury. What would have been a rather alarming effect is therefore probably spurious, and might be summarized with the suggestion that birth injuries occur no more frequently during weekend deliveries, but are more reliably reported on the birth certificates. One of the most striking conclusions that one must come to from such studies is the very low reliability of information collected in such a routine fashion from birth certificates.

Also in progress is a detailed analysis of a comprehensive survey of child health which embraced virtually every family in Santa Clara County.

Assertions are frequently made to the effect that "3% of the population of the United States" is afflicted with mental retardation, but these are only the best "guestimates" that can be made at the present time and much more intensive demographic study is required to establish the precise

magnitude of the problem. While our present standards of coping with mental retardation as a public health problem are so poor, it may not be as consequential to make such precise measurements. However, as we begin to come close to dealing with the problem, intelligent planning will require better than an order of magnitude accuracy in our estimates.

Biochemical Studies on Brain Proteins and Developmental Neurology.

Drs. E. Shooter, H. Posner and J. Lederberg.

Dr. Shooter for the last year was on leave at the University of London, where he was continuing his biochemical work on the protein composition of subcellular fractions of nervous tissue. During the year Dr. Posner was a Visiting Fellow from the National Institutes of Health, and together with Miss Nancy Martin, helped to organize our laboratory for studies on brain proteins. Methods have been established for the fractionation of brain proteins on the acrylamide gel with electrophoresis. Perhaps the most notable finidng was a method for the solublization of otherwise insoluble proteolipids by initial treatment with hydrazine. This technique opens up the possibility of a further fractionation and characterization of the structural proteins of the brain which should be especially pertinent for pathological and developmental studies.

Developments in Instrumentation for Ultrastructural Biochemistry.

The state of biochemistry at the finest levels faces very difficult problems in the analysis of structures which are easily visible under the microscope or electron microscope, but whose chemical composition may be extremely difficult, though important to determine. As a new approach to this problem we are undertaking the construction of a scanning mass spectrometer which is designed to furnish a mass spectrometric analysis of every point on a specimen of which a photomicrograph could be taken.

PUBLICATIONS

1) Kretchmer, N. "Whither - Birth Defects". Perspectives in Biology and Medicine - in press.

2) Winick, M. and Greenberg, R.E. Appearance and Localization of a Nerve Growth-Promoting Protein During Development. Pediatrics in press.

3) Winick, M. and Greenberg, R.E. Chemical Control of Sensory Garglia During A Citical Period of Development Science. in press.

4) Petersen, C.D. and Luzzatti, L. The Role of Chromosome Translocation on the Recurrence Risk in Down's Syndrome. Pediatrics in press.

5) McKhann, G.M. and Moser, H.W. Metachromatic Leucodystrophy Kennedy Foundation Symposium. 1964.

6) McKhann, G.M., Ho, W., and Levy, R. The Stimulation of Sulfatide Synthesis by Exogenous Cerebroside. submitted to Nature.

ABSTRACTS

1) Petersen, C.D. and Luzzatti, L. Chromosome Studies of Children with Down's Syndrome Born of Mothers Below 30 Years of Age. American Pediatric Society, 1963.

2) McKhann, G.M., Moser, H.W. and Moser, A. Sulfate Metabolism in Metachromatic Leukodystrophy. Society for Pediatric Research. 1964.

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